Floral biology of the Western Australian endemic 'yellow bells', *Geleznowia verrucosa* Turcz (Rutaceae)

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Abstract

Aspects of the floral biology of the two subspecies of *Geleznowia verrucosa* and one intermediate population were examined for possible differences in their reproductive strategy(ies). Movement of reproductive organs, stigma receptivity prior to anther dehiscence, and an apparent lack of pollinators indicate that *G. verrucosa* is a protogynous facultative selfer. Hand-pollination treatments confirm that although self-pollination does produce seed, higher levels of seed are set when flowers are pollinated with pollen outcrossed from within the population. Differing responses by the taxa to the various pollination treatments suggests that contrasting reproductive strategies are employed. The ssp *verrucosa* ms is more oriented towards self-pollination while ssp *formosa* ms has characteristics of a mixed mating system. Responses of the intermediate population suggest that it may be a hybrid.

Keywords: flower, reproductive organs, pollination, *Geleznowia verrucosa*

Introduction

Plant reproductive strategies play an important role in gene transmission, population genetic structure, selection response, and speciation (Brown & Allard 1970; Grant 1981; Lyons & Antonovics 1991; Ellis & Sedgley 1992; Barrett *et al.* 1996). Evolutionary shifts in the breeding strategies of plants are usually associated with a move from obligate outbreeding to predominantly self-fertilisation, although reverse shifts are known (Stebbins 1970; Gottlieb 1973; Jain 1976). These shifts are often correlated with changes in floral morphology and resource allocation. Selfing populations frequently exhibit smaller petals, stamens and styles, reduced nectar and pollen production, and lower pollen:ovule ratios, essentially investing fewer resources in floral attractants while setting more seed (Jain 1976; Ritland & Ritland 1989). In contrast, predominantly outcrossing species possess larger and more abundant flowers, but set a lower proportion of fruit and seeds (Lyons & Antonovics 1991).

The 'yellow bells', *Geleznowia verrucosa* Turcz (Rutaceae), is a small woody endemic Western Australian species characterised by terminal clusters of bright yellow flowers. The genus is widely distributed across Western Australia, extending from Cape Range (21° 50′ S 116° 12′ E) in the north of Western Australia, to Dowerin (31° 12′ S 117° 02′ E) in the eastern wheatbelt (Hnatiuk 1990; Keighery & Gibson 1993). Although once considered monospecific, recent allozyme and morphometric studies have confirmed the existence of two subspecies and a series of intermediate populations (Fig 1), which are thought to be of ancient hybrid origin (Broadhurst *et al.* 1999; Broadhurst 2000). Genetically, the smaller ssp *verrucosa* (manuscript) is allied with the intermediate populations but morphologically the intermediate populations are more similar to the larger ssp *formosa* (ms, Broadhurst *et al.* 1999; Broadhurst 2000). Formal revision of the genus is being undertaken.

Like many Western Australian plant genera, little is known about the reproductive biology of *G. verrucosa*. An understanding of the reproductive mechanisms employed was considered essential for interpreting patterns of genetic and morphological variation. The presence of reproductive isolating mechanisms would also provide further evidence of divergence between the taxa. Aspects of the floral biology and the results of pollination experiments designed to determine the reproductive strategy(ies) employed by *G. verrucosa* are reported here.

Materials and methods

Sampling strategy

Experimental populations were selected from across the species distribution to include two populations of the subspecies *verrucosa* ms and *formosa* ms and one population of the intermediate form (Table 1). Although a population of ssp *verrucosa* occurred in the north of this subspecies' range at Indarra Nature Reserve, flowering was poor, limiting experimental populations to Arinya and Coorow located in the south. All sampling and pollination experiments were undertaken during 1995 and 1996.

Flowering events, phenology and pollinators

Flowering events were observed in the field and the laboratory. In the field, flowers were tagged on 3 to 4 plants in each population, and events from flower opening to closing were recorded and photographed. Stems of inflorescences were also transported to the laboratory, maintained in vases of water and floral events observed, recorded and photographed.

The commencement and completion of flowering at the five populations was recorded during monthly visits to the sites. Opportunistic day-time and nocturnal obser-

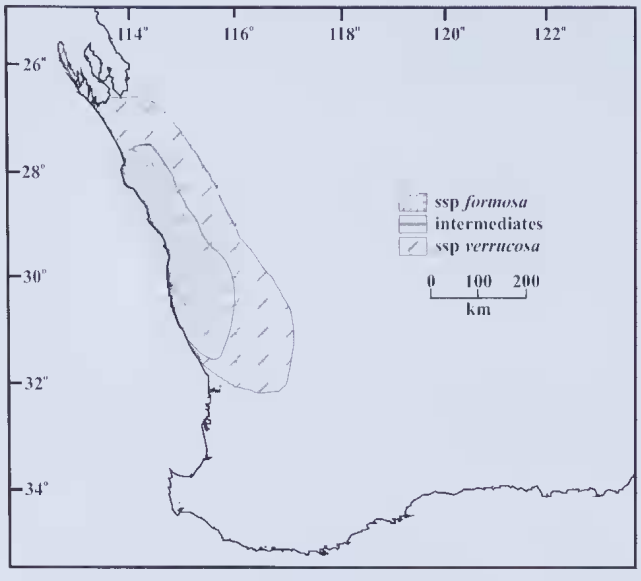

Figure 1. Distribution of *Geleznowia verrucosa* specimens recorded at the WA Herbarium and mapped using WABIOTA. Data correct at 14 July 2000.

vations were also undertaken during these visits with the type and activity of invertebrates on the plants noted.

Stigma receptivity

Stigmas from buds and flowers of various ages were examined both in the field and the laboratory. To determine timing and length of receptivity, pistils from buds and flowers of varying ages were detached from the ovary and placed in 3% hydrogen peroxide (Galen & Kevan 1980; Dafni 1992). Stigmas which are receptive should produce bubbles (Dafni 1992). The size, abundance and distribution of bubbles from the stigmatic surface were qualitatively assessed.

Pollen viability and pollen tube growth

Stems with inflorescences were collected from each population, placed in sealed plastic bags, stored at 4 °C and transported to the laboratory. Flowers were allowed to open naturally and dehiscing anthers were collected, bulked and pollen viability immediately determined. Attempts to indirectly measure pollen viability by germinating pollen grains in a suitable sucrose solution (Dafni 1992) were unsuccessful. Instead, viability was assessed by staining pollen grains with either Alexander's stain (Alexander 1980) or fluorescein diacetate (Heslop-Harrison & Heslop-Harrison 1970). Two hundred pollen grains were scored as either red (viable) or green (non-viable) following Alexander's stain while 500 pollen grains were scored as either bright (viable) or non-stained (non-viable) following staining with fluorescein diacetate and viewed with a violet exciter filter (λ = 395-415 nm).

Table 2. Pollen source for experiments observing pollen tube growth.

	Arinya	Geraldton	Meanarra Hill 1
Maternal	ssp verrucosa	ssp formosa	ssp formosa
x	x	x	x
Paternal	ssp formosa	ssp verrucosa	intermediate
Pollen Source	Meanarra Hill 1	Indarra Reserve	Nearby population

To determine whether pollen tube growth was influenced by pollen source, 4 to 5 flowers on five plants were hand-pollinated using pollen from within the population. A further 4 to 5 flowers on these plants were hand-pollinated with pollen sourced from a different taxon (Table 2). Hand-pollinations were conducted by wiping mature dehiscing anthers across the stigmatic surface of emasculated receptive flowers until pollen was clearly visible. The flowers were collected after 24 h, fixed in formalin:proprionic acid:ethanol (40:50:10 v/v) and stored at 4 °C in 70% ethanol. The pistils were later autoclaved in sodium sulphate (50 g L^{-1}) for 30 min at 121 °C, stained with decolourised aniline blue for 24 h, and viewed under fluorescent light using a violet exciter filter (λ = 395-415 nm; Martin 1958; Dafni 1992). Stigmas and styles were examined for pollen germination, pollen tube growth and abnormalities. Due to the limited number of receptive flowers available for both this and the pollination experiment, pollen tube growth could not be assessed at either Coorow 1 and Hutt River.

Pollination experiments

Field pollination experiments were conducted during 1996. Newly opened flowers with undehisced anthers were manipulated as outlined in Table 3. Hand pollinations were undertaken by wiping dehiscing anthers over receptive stigmas until pollen was clearly visible. Differences in flowering times necessitated pollen for the cross pollination treatment between populations (X_{OTHER}) at the two ssp *verrucosa* ms sites being stored until experiments could be conducted. The pollen was stored in dark, cool conditions and viability was assessed using fluorescein diacetate prior to use. Transfer of pollen to stigmas was facilitated using a fine hair brush. Potential pollinators were excluded from flowers by tying a nylon stocking bag over the inflorescences and applying a clear sticky substance (Bird-Off, Rentokil) around the stem below the tie. To prevent seed from treated and untreated flowers mixing, the fruit of treated flowers were collected before maturity and seed

Table 1. Location of sampled *G. verrucosa* populations; n_{est} is the estimated population size.

Population	Taxon	n_{est}	Latitude (°S)	Longitude (° E)
Arinya	ssp *verrucosa*	50	31° 19′ 52″	116° 58′ 36″
Coorow 1	ssp *verrucosa*	20	29° 54′ 08″	116° 00′ 05″
Hutt River 2	intermediate	200	28° 05′ 28″	114° 28′ 02″
Geraldton	ssp *formosa*	24	28° 35′ 25″	114° 37′ 57″
Meanarra Hill 1	ssp *formosa*	25	27° 41′ 40″	114° 13′ 00″

Table 3. Pollination treatments undertaken for *G. verrucosa* populations (after Dafni 1992).

	Test	Treatment
Open	Open-pollination	Untreated, unbagged
Selfed	Self-pollination	Untreated, bagged
X_{SAME}	Cross-pollination within populations	Emasculated, pollen from within site
X_{OTHER}	Cross-pollination between populations	Emasculated, pollen from outside site
No pollen	Non-sexual	Emasculated, bagged

set assessed. Although the fruits were green, the seeds were well formed and empty carpels were clearly distinguishable.

One-way Analyses of Variance (ANOVA) were conducted using SuperANOVA (Abacus Concepts 1989) to determine whether treatments varied within and between populations. Means were compared using Tukey's Compromise (Tukey's b; Winer 1962) *post hoc* test. To test for possible bias due to the large differences in values between open-pollinated and the remaining treatments, the former values were removed, the ANOVA conducted again and contrasts between means re-assessed.

To assess the effects of the cross-pollination treatments, an index of self-incompatibility (ISI = fruit set from self-pollination/fruit set from cross-pollination; Zapata & Arroyo 1978; Kenrick 1986) was calculated for both pollination treatments (X_{SAME} and X_{OTHER}). This calculation indicates the compatibility of pollen to set seed. Values of <0.2 indicate self-incompatibility, 0.2-0.9 partial self-compatibility, 1.0 self compatibility and >1.0 preferential self-pollination (Zapata & Arroyo 1978; Kenrick 1986).

Results

Flowering events, phenology and pollinators

The inflorescences of *G. verrucosa* are comprised of a complex suite of developmental stages from bud to fruit. Mature flowers consist of several large yellow bracts surrounding five sepals of similar shape and colour, with five smaller, thinner and darker yellow petals inside (Fig 2). Mature buds generally open within a short space of time during early morning, although this may be delayed on overcast days. The flowers remain open for a single day. The sequence of flowering events begins as the bud bursts open, splaying petals outwards; the sepals are already extended outwards at this time (Fig 2, parts 1-3). Upon opening, the long style, bent over in the closed bud, is released, and straightens. The style and stamens move away from each other and by mid-morning are at their furthermost (Fig 2, part 4). The anthers now dehisce, limiting the possibility of self pollen falling on its own stigma. After midday, the style and stamens begin to move towards each other and by mid-afternoon, are close together (Fig 2, part 5). The petals begin to close in the late afternoon, enclosing both pistil and stamens (Fig 2, part 6).

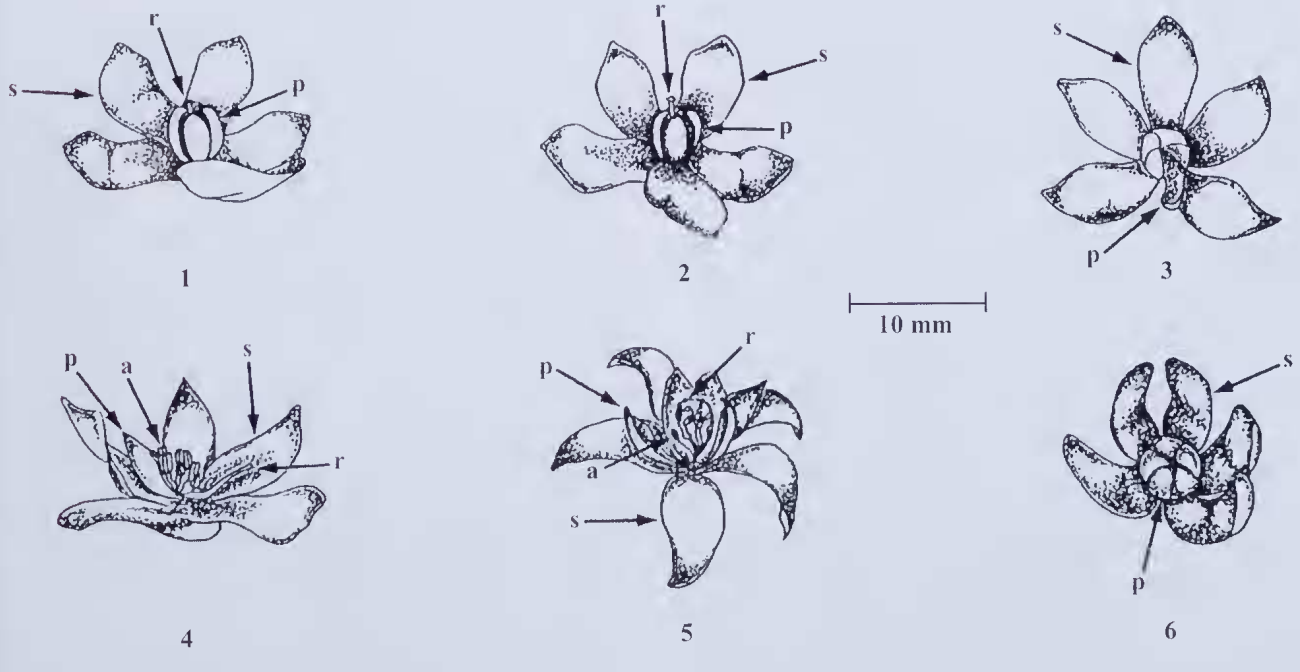

Figure 1. Flowering stages for *G. verrucosa* (drawings by E Ladhams). a, anthers; p, petals; r, style; s, sepals.

Table 4. Flowering seasons for all *G. verrucosa* populations over two years.

Population	Taxon	Flowering season	
		1995	1996
Arinya	ssp *verrucosa*	Sept - Oct	Aug - Nov
Coorow 1	ssp *verrucosa*	Aug - Oct	Sept - Nov
Hutt River 2	intermediate	May - Aug	May - July
Geraldton	ssp *formosa*	July - Oct	Aug - Oct
Meanarra Hill 1	ssp *formosa*	June - Sept	July - Aug

While all taxa exhibited a similar pattern of flowering phenology, separation between the pistils and anthers of ssp *verrucosa* ms did not appear to be as pronounced as that observed in ssp *formosa* ms.

Commencement and length of flowering differed between populations and years (Table 4). Flowering in the ssp *verrucosa* ms populations commenced later (August/September) than in the ssp *formosa* ms populations (June/July). In contrast, flowering in the intermediate population at Hutt River 2 was earliest in both years (May). The 1995 flowering period was longer than that experienced during 1996.

Despite obvious floral attractants such as large yellow bracts and prominent oil glands, no significant diurnal or nocturnal pollinators were observed visiting *G. verrucosa* plants during flowering. A small moth (Lepidoptera) was occasionally observed during the day and larvae found in some buds suggests that flowers act as a nursery. Ants (Hymenoptera) were noted apparently removing pollen on two plants at the Geraldton site. Although the ants did not appear to contact the style, it is possible that some accidental pollination does occur. Small beetles (Coleoptera) were also observed during flowering. The low number of insects observed suggests that none are significant pollinators of *G. verrucosa*.

Stigma receptivity

In the tests for stigma receptivity, several small bubbles were observed on the stigmatic surface when buds were green, or yellow-green and unopened, indicating that receptivity to pollen was weak. The stigmatic surface of buds which were yellow and just opened produced larger bubbles, suggesting increased receptivity. The stigmas of open flowers in which 0-50% of the anthers were dehisced produced many large bubbles, which increased in number when all anthers were dehisced and as the flower was closing, indicating greater pollen receptivity.

Pollen viability and pollen tube growth

All populations exhibited similarly high pollen viability (>90%) for both the Alexander's and fluorescein diacetate stains. Little misshapen or abnormal pollen was observed for any population.

All pollen tubes grew in the stylar tissue towards the ovary regardless of pollen source and no abnormal pollen tube growth was observed. While pollen tube growth in pollinated flowers from Geraldton and Meanarra Hill 1 extended the length of the style, at Arinya pollen tube growth was not as pronounced. Too few flowers were available at Coorow 1 and Hutt River for this experiment.

Pollination experiments

Within populations no significant variation was noted between plants for any of the treatments, allowing the data to be pooled. The experimental populations responded differently to the treatments (Table 5). All populations, except Geraldton, set significantly more open-pollinated seed than for any other treatment. At Geraldton, open-pollinated seed set was comparable to that of both cross-pollination treatments. While no variation occurred between the remaining treatments at Arinya and Meanarra Hill 1, the selfed and X_{SAME} treatments at Coorow produced more seed than the X_{OTHER} and pollen exclusion treatments. Self-pollination in the Hutt River 2 population produced more seed than both cross-pollination treatments; insufficient flowers were available for the pollen exclusion treatment at this population. Removing the open-pollinated values from the analysis did not significantly change these patterns.

Comparisons of treatments between populations highlighted several important differences (Table 5). Open-pollination seed set at Geraldton and Meanarra Hill 1 was considerably lower than in the other populations ($F_{4,163} = 19.56$; $P = 0.0001$), while significantly more self-pollinated seed was set at Hutt River 2 ($F_{4,150} = 12.10$; $P = 0.0001$). The X_{SAME} treatment produced similarly high levels of seed set at Coorow 1 and Geraldton and low levels at Arinya and Meanarra Hill 1, with intermediate levels set at Hutt River 2 ($F_{4,143} = 9.17$; $P = 0.0001$). Low seed set occurred following the X_{OTHER} treatment at Arinya, Coorow 1 and Meanarra Hill 1 ($F_{4,128} = 11.39$; $P = 0.0001$).

There was substantial variation in ISI values between populations (Table 6). The Arinya and Coorow 1 populations were partially self-compatible when pollinated with X_{SAME} pollen, but preferentially self-pollinating when pollinated with X_{OTHER} pollen sourced from the ssp *formosa* ms. The intermediate population at Hutt River 2 was preferentially self-pollinating regardless of pollen source, while the ssp *formosa* ms populations were partially self-compatible irrespective of pollen source.

Although populations responded to the various pollination treatments differently, some trends were apparent. To investigate this further, data for the different subspecies were pooled and compared (Fig 3). The ssp *formosa* ms set considerably less open-pollinated seed than either ssp *verrucosa* ms or the intermediate population. Seed set from selfing was highest in the intermediate population and lowest in ssp *formosa* ms, while all populations set similar levels of seed following X_{SAME} pollination. The X_{OTHER} treatment elicited similar levels of seed set in ssp *formosa* ms and the intermediate population but significantly lower

Table 5. Tukey's Compromise comparison of mean seed set per flower for pollination treatments within and between populations. Same letter within columns indicates no significant difference. -, insufficient flowers; number in parentheses. *P=0.001, **P=0.0001.

Within populations	Population				
Treatment	Arinya	Coorow 1	Hutt River 2	Geraldton	Meanarra Hill 1
Open	$3.42^{a}_{b} \pm 0.21$ (33)	$4.03^{a}_{b} \pm 0.15$ (31)	$4.33^{a}_{b} \pm 0.18$ (27)	$2.00^{a}_{b} \pm 0.27$ (47)	$2.30^{a}_{b} \pm 0.26$ (30)
Selfed	$0.88^{b} \pm 0.29$ (25)	$1.68^{b} \pm 0.36$ (25)	$3.05^{b} \pm 0.46$ (19)	$0.98^{d} \pm 0.22$ (53)	$0.12^{b} \pm 0.12$ (33)
X_{SAME}	$0.80^{b} \pm 0.26$ (30)	$2.19^{c} \pm 0.29$ (27)	$1.33^{b} \pm 0.56$ (15)	$2.37^{a} \pm 0.25$ (46)	$0.53^{b} \pm 0.21$ (30)
X_{OTHER}	$0.16^{b} \pm 0.16$ (25)	$0.09^{c} \pm 0.09$ (23)	$1.58^{b} \pm 0.50$ (19)	$1.96^{a} \pm 0.26$ (44)	$0.50^{b} \pm 0.18$ (22)
No pollen	$0.80^{b} \pm 0.53$ (10)	$0.07^{c} \pm 0.05$ (28)	-	0^{c} (33)	0^{b} (20)
F_{df} P	$28.67_{4,118}$ **	$59.98_{4,129}$ **	$12.96_{3,76}$ **	$14.22_{4,218}$ **	$25.42_{4,130}$ **

Between Populations	Treatment				
Population	Open	Selfed	X_{SAME}	X_{OTHER}	No pollen
Arinya	$3.42^{b}_{ab} \pm 0.21$ (33)	$0.88^{bc}_{b} \pm 0.29$ (25)	$0.80^{b}_{a} \pm 0.26$ (30)	$0.16^{b}_{b} \pm 0.16$ (25)	$0.80^{a}_{b} \pm 0.53$ (10)
Coorow 1	$4.03^{a}_{a} \pm 0.15$ (31)	$1.68^{a}_{b} \pm 0.36$ (25)	$2.19^{a}_{ab} \pm 0.29$ (27)	$0.09^{a}_{b} \pm 0.09$ (23)	$0.07^{a}_{b} \pm 0.05$ (28)
Hutt River 1	$4.33^{a}_{c} \pm 0.18$ (27)	$3.05^{a}_{bc} \pm 0.46$ (19)	$1.33^{a}_{ab} \pm 0.56$ (15)	$1.58^{a}_{a} \pm 0.50$ (19)	-
Geraldton	$2.00^{c}_{a} \pm 0.27$ (47)	$0.98^{bc}_{b} \pm 0.22$ (53)	$2.37^{a}_{b} \pm 0.25$ (46)	$1.96^{a}_{b} \pm 0.26$ (44)	0^{b}_{b} (33)
Meanarra Hill 1	$2.30^{c}_{a} \pm 0.26$ (30)	$0.12^{c}_{b} \pm 0.12$ (33)	$0.53^{b}_{b} \pm 0.21$ (30)	$0.50^{b}_{b} \pm 0.18$ (22)	0^{b}_{b} (20)
F_{df} P	$19.56_{4,163}$ **	$12.10_{4,150}$ **	$9.17_{4,143}$ **	$11.39_{4,128}$ **	$5.75_{3,87}$ *

Table 6. Index of self-incompatibility (ISI) for the cross pollination treatments in *G. verrucosa* populations. ISI values: <0.2 self-incompatibility, 0.2-0.9 partial self-compatibility, 1.0 self compatibility and >1.0 preferential self-pollination. l, partially self-compatible; u, preferential self-pollination.

Pollen source	Population	maternal	x	paternal	ISI	
X_{SAME}	Arinya	ssp *verrucosa*	x	ssp *verrucosa*	0.92	l
	Coorow 1	ssp *verrucosa*	x	ssp *verrucosa*	0.78	l
	Hutt River 2	intermediate	x	intermediate	2.65	u
	Geraldton	ssp *formosa*	x	ssp *formosa*	0.52	l
	Meanarra Hill 1	ssp *formosa*	x	ssp *formosa*	0.25	l
X_{OTHER}	Arinya	ssp *verrucosa*	x	ssp *formosa*	5.50	u
	Coorow 1	ssp *verrucosa*	x	ssp *formosa*	21.00	u
	Hutt River 2	intermediate	x	ssp *formosa*	1.77	u
	Geraldton	ssp *formosa*	x	ssp *verrucosa*	0.66	l
	Meanarra Hill 1	ssp *formosa*	x	intermediate	0.36	l

seed was produced by ssp *verrucosa* ms. This subspecies also set a small amount of seed despite pollen exclusion.

Discussion

The movement of reproductive organs during flower opening and closing, stigma receptivity prior to anther dehiscence, and the apparent absence of pollinators suggest that *G. verrucosa* is a protogynous facultative selfer. Seed production following hand pollination supports this premise. In all populations except the intermediate population at Hutt River 2, the levels of seed set were similar to, or higher, in flowers that were outcrossed within the population, compared with flowers that were allowed to naturally self-pollinate. The ISI values further reflect that these populations are partially, rather than completely, self-compatible. It would appear that although *G. verrucosa* plants were more receptive to outcrossed pollen, seed set can still occur when this pollen is unavailable. Seed set following pollination between the various taxa elicited various responses.

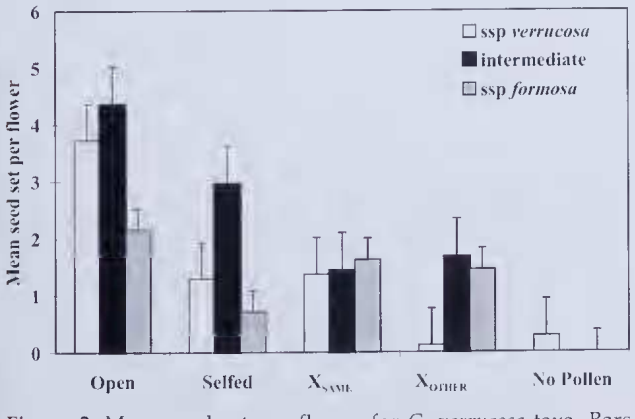

Figure 3. Mean seed set per flower for *G. verrucosa* taxa. Bars indicate one standard error

Both the ssp *verrucosa* ms populations set considerably fewer seed when pollinated with pollen from ssp *formosa* ms, and had ISI values consistent with preferential self-pollination. This result may reflect a decline in pollen viability due to storage. However, since storage did not affect pollen viability, it is more plausible that some other mechanism is responsible for this result. In contrast, the two ssp *formosa* ms populations had partial self-compatible ISI values when crossed with either a ssp *verrucosa* ms or the intermediate population. Interestingly, although pollen sourced from outside the population elicited the same response in ssp *formosa* ms, more seed was set following pollination with ssp *verrucosa* ms pollen than with pollen from the intermediate population. A lack of abnormalities in pollen tube growth suggest that these responses

probably result from post- rather than pre-zygotic barriers to fertilisation. The responses elicited by the two subspecies suggest that different reproductive the strategies are employed.

While both populations of the two subspecies exhibited partial self-compatibility ISI values when outcrossed within the population, the values for ssp *verrucosa* ms were considerably higher. Given that these populations were preferentially self-pollinating when crossed from outside populations, it would appear that this taxon is more oriented towards selfing than ssp *formosa* ms. The production of some seed in crosses with a non-preferred pollen source however, indicates that reproductive isolation is not complete and some ability to outcross with related taxa has been preserved. The similar levels of seed set by ssp *formosa* ms regardless of pollen source, are suggestive of a mixed mating system. Although an allozyme study undertaken to confirm these differences failed to find sufficient polymorphism for a complete analysis, observations did confirm that ssp *formosa* ms had a mixed mating system (Broadhurst 1998).

The differences in timing and length of flowering between the *G. verrucosa* taxa may also be indicative of reproductive differences. The later flowering of the two ssp *verrucosa* ms populations may reflect an ecological gradient as flowering moves across the south-west botanical district from the north-west to the south-east. Indeed, in a population approx 50 km south of Kalbarri where both subspecies co-occur, some overlap in flowering has been noted (Broadhurst 1998). However, this population appears to be a hybrid zone where the introgression of genetic material from ssp *formosa* ms to ssp *verrucosa* ms has occurred, possibly explaining the flowering overlap (Broadhurst *et al.* 2001). At another site located to the east of Kalbarri, several ssp *verrucosa* ms plants co-occur with an intermediate population. Flowering patterns here are quite distinct with the intermediate plants finishing prior to ssp *verrucosa* ms commencing flowering (Broadhurst 1998) suggesting that some underlying genetic, rather than ecological component, influences flowering patterns.

Seed set in the intermediate population at Hutt River 2 was not consistent with trends observed in either of the two subspecies. Since evolutionary shifts in breeding strategy can be mediated through a series of mixed mating systems (Stebbins 1957), this population could represent a transitional form between ssp *formosa* ms and ssp *verrucosa* ms. The high levels of natural seed set, significantly higher selfing rates, and strong preference for self-pollination in the intermediate population suggest that this is not a transitional form. The Hutt River 2 population belongs to a group of populations thought to be of ancient hybrid origin (Broadhurst *et al.* 1999) and as such a selfing mechanism would ensure reproductive success in the event of reproductive isolation from parental types.

Differences in breeding strategies are themselves not necessarily indicative of systematic separation. However, speciation often follows events which prevent gene flow and promote strong natural selection (Grant 1981). The ssp *verrucosa* ms is widely distributed with many populations occurring in the transitional-rainfall zone, a region characterised by the evolution of small, disjunct populations (Hopper 1992). When pollen movement is limited, morphological and physiological traits which promote selfing may be favoured, particularly where plant density is low or pollinators scarce (Jain 1976; Lloyd 1980; Schemske & Lande 1985). Self-fertilisation is commonly associated with ecological radiation into temporary, pioneer habitats (Stebbins 1957; Jain 1976; Lloyd 1980). The ssp *verrucosa* ms occurs in drier, more marginal habitats, and a shift towards selfing would ensure reproductive success in a difficult and unpredictable environment, particularly given the periodic drought experienced in the transitional rainfall zone. Retaining some ability to outcross may be significant during range expansions and contractions facilitated by environmental fluctuation, and lead to gene exchange between populations and taxa.

Reproductive strategy(ies) vary within the *G. verrucosa* taxa. The ssp *formosa* ms employs a mixed-mating system while ssp *verrucosa* ms is more oriented towards a selfing strategy. The intermediate population exhibited a strong self-compatible mechanism, suggesting that it may be the product of hybridisation between the two subspecies.

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